Data Evaluation Report on the Acute Toxicity of Bifenthrin to Freshwater Amphipods (*Hyalella azteca*)

D425863 EPA MRID Number 49552201

Data Requirement: EPA Guideline 850.1020 Acute Toxicity to Freshwater Invertebrate

Test material: Bifenthrin technical **Purity:** 93.6%
Chemical name: IUPAC: 2-methylbiphenyl-3-ylmethyl (1*RS*,3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate
CAS name: 2-methyl[1,1'-biphenyl]-3-yl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.: 82657-04-3
Synonyms: FMC 54800

Primary Reviewer: Christie E. Padova **Signature:** Christie E. Padova
Staff Scientist, CSS-Dynamac **Date:** 07/07/15

Secondary Reviewer: John Marton, Ph.D. **Signature:**
Environmental Scientist, CDM Smith **Date:** 07/07/15

Primary Reviewer: Brian Montague, Fishery Biologist **Date:** August 03. 2015
ERB5/EFED/OPP/OCSPP

EPA PC Code 128825
EPA DP Barcode 425863
EPA MRID 49552201

Date Evaluation Completed: August 4, 2015

CITATION: Bradley, M.J. 2013. Bifenthrin – Acute Toxicity to Freshwater Amphipods (*Hyalella azteca*) Under Flow-Through Conditions. Unpublished study performed by Smithers Viscient, Wareham, MA. Laboratory Study No. 13656.6164. Study sponsored by Pyrethroid Working Group, FMC Corporation, Ewing, NJ. Study initiated March 27, 2012 and completed September 20, 2013.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study or to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of bifenthrin to 9-day old *Hyalella azteca* was studied under flow-through conditions. Amphipods were exposed to bifenthrin technical at nominal concentrations of 0 (negative and solvent controls), 0.25, 0.50, 1.0, 2.0 and 4.0 ng/L. Mean-measured concentrations were <0.056 (<LOQ, controls), 0.28, 0.53, 1.0, 2.2 and 4.7 ng ai/L.

In the negative control, solvent control, and mean-measured 0.28, 0.53, 1.0, 2.2 and 4.7 ng ai/L treatment levels, mortality averaged 0, 0, 0, 15, 25, 85 and 80%, respectively, after 24 hours; 0, 0, 0, 30, 70, 95 and 100%, respectively, after 48 hours; 0, 0, 5, 55, 85, 100 and 100% respectively, after 72 hours; and 0, 0, 5, 55, 100, 100 and 100%, respectively, after 96 hours. The 96-hr LC_{50} (with 95% C.I.) was 0.493 (0.419 to 0.580) ng ai/L. Erratic swimming followed by lethargy and/or immobility were observed in (several to all) amphipods from the ≥0.53 ng ai/L levels during the study.

Bifenthrin would be classified as **very highly toxic** to *Hyalella azteca* in accordance with the classification system of the U.S. EPA.

This study classified as **acceptable** and may be used in Agency risk assessments.

Results Synopsis

Test Organism Age (e.g., 1st instar): 9 days old
Test Type (Flow-through, Static, Static Renewal): flow-through

96-hour

LC_{50}: 0.493 ng ai/L	95% C.I.: 0.419-0.580 ng ai/L
Probit Slope: 7.32	95% C.I.: 4.18-10.5

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on the procedures outlined in the U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.1020, Gammarid Acute Toxicity Test (*draft*, 1996) and U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.1000, Special Considerations for Conducting Aquatic Laboratory Studies (*draft*, 1996).

Notable deviations from OPPTS *draft* 850.1020 guidance (1996) include:

- Although data were available, the 24- through 72-hour LC_{50} values and associated 95% C.I. were not reported.
- Amphipods were fed daily during testing.
- Although *Hyalella azteca* is not a currently a standard test species for freshwater invertebrate acute toxicity testing, this study was requested as part of Regulation Review due to knowledge of its enhanced sensitivity to pyrethroids relative to *Daphnia*. Test protocol was submitted prior to the conduct of testing.

COMPLIANCE:
Signed and dated Data Confidentiality, Quality Assurance, and GLP compliance statements were provided. The study was conducted in accordance with U.S. EPA GLP standards (40 CFR Part 160), with the following exceptions: routine water and food contaminant screening analyses were not performed according to GLP, but were performed in a certified laboratory using standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test material:	Bifenthrin technical
Description:	Not reported
Lot No./Batch No. :	PL09-0251
Purity:	93.6%
Stability of compound under test conditions:	Stable
Storage conditions of test chemicals:	Room temperature, in original container

Physicochemical properties of bifenthrin.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species: *Hyalella azteca*
Age at test initiation: 9 days old
Source: Laboratory cultures maintained at Smithers Viscient (Wareham, MA).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: In a 96-hr range-finding study, amphipods (20/level) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.50, 1.0, 2.0, 4.0 and 8.0 ng/L under flow-through conditions. Following 96 hours of exposure, mortality was 0% in the negative and solvent control levels, and 5, 55, 90, 100 and 100% among amphipods exposed to the 0.50, 1.0, 2.0, 4.0 and 8.0 ng/L treatment levels, respectively. All surviving amphipods exposed to the 1.0 ng/L treatment level were observed to be lethargic, while all surviving amphipods exposed to the 2.0 ng/L level were observed to be immobilized. Concentrations selected for use in the definitive study were based on these results and in consultation with the Sponsor.

b. Definitive study:

Table 1: Experimental Parameters

Parameter/Criteria	Details	Remarks
Acclimation *The recommended acclimation period is a minimum of 7 days* Period: Conditions (same as test or not): Feeding: *Organisms should not feed during the study.* Health (any mortality observed): *Pretest mortality should be < 3% 48 hours prior to testing.*	9 days Same During holding, amphipods were fed with a combination of yeast, cereal leaves and flaked fish food suspension (YCT), as well as unicellular green algae (*Ankistrodesmus falcatus*). Appeared healthy and no mortalities were observed 48 hours prior to initiation.	Sexually-mature amphipods were isolated from the main culture 10 days prior to initiating the test and young produced by these organisms (the following day) were pipetted into 1 L beakers containing *ca.* 0.90 L dilution water. During the 9-day holding period, DO ranged from 7.3 to 7.8 mg/L and temperature ranged from 24 to 25°C. Each replicate chamber received 1.0 mL of YCT once daily during the 96-hr exposure period.
Duration of the test: *EPA requires 96 hours, except daphnids which are 48 hours.*	96 hours	
Test conditions Static/flow-through: Type of dilution system for flow- through method: Renewal rate : *The recommended flow rates are 5 - 10 volume additions 24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*	Flow-through Intermittent-flow proportional diluter N/A	Flow-rate provided *ca.* 10 volume additions per day, with a 90% replacement time of *ca.* 5 hours. The exposure system was properly operating for 3 days prior to exposure to allow equilibration of the test substance in the diluter system.
Aeration, if any:	None	

Parameter/Criteria	Details	Remarks
Test vessel Material (glass/stainless steel): Size: Fill volume: *EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution*	Glass beakers affixed with 40-mesh Nitex® screens over the slot 2 L 1.8 L	Each test vessel contained a 3-cm^2 piece of 250-µm stainless steel mesh as a substrate.
Source of water: *Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water Dilution water should be intensely aerated before the study.*	The dilution water used during the study was laboratory well water	

Parameter/Criteria	Details	Remarks
Water parameters Hardness: *EPA recommends 40 - 48 mg L as $CaCO_3$ (OECD recommends 140 - 250 mg L)*	28 mg/L as $CaCO_3$	It was reported that representative samples of the dilution water source were analyzed periodically for the presence of pesticides, PCBs and toxic metals by Geol.abs, Inc. (Braintree, MA). None of these compounds were detected at concentrations considered toxic.
pH: *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations*	7.3 to 7.7	7.8-8.2 recommended for Hyalella or Chironomid testing
Dissolved oxygen: *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*	7.4 to 8.8 mg/L (≥75% saturation)	
Total Organic Carbon:	1.5 mg C/L (March 2013)	
Particulate matter:	Not reported	
Metals:		
Pesticides:		
Chlorine:		
Temperature: *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22°C (±1°C)	Daily: 22 to 24°C Continuous: 22 to 24°C	Common temperature range for naturally occurring Hyalella azteca (20-30°C)
Other parameters:	Conductivity: 94 to 100 µS/cm Alkalinity: 26 to 28 mg/L as $CaCO_3$	
Intervals of water quality measurement:	Dissolved oxygen, temperature, and pH were measured in Replicate A of each treatment level and controls at test initiation and in alternating replicates daily thereafter. Additionally, temperature was measured continuously in Replicate B of the nominal 2.0 ng/L treatment level. Total hardness, alkalinity, and conductivity were measured in a composite sample of each treatment level and control at the beginning of the study.	

Parameter/Criteria	Details	Remarks
Number of replicates Negative control: Solvent control: Treated: *EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels*	 2 2 2 per level	
Number of organisms/replicate Negative control: Solvent control: Treated: *EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *If a limit test is conducted, it must be shown that the LC_{50} EC_{50} is >100 mg/L by exposing ≥30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and ≤ 0.5 g/L at > 17°C; flow-through: ≤ 10 g/L at ≤ 17°C and ≤5 g/L at > 17°C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*	 10 10 10	

Parameter/Criteria	Details	Remarks
Treatment concentrations Nominal: Mean-measured: *Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g L, while the lowest concentration should have no observable effect.*	0 (negative and solvent controls), 0.25, 0.50, 1.0, 2.0 and 4.0 ng/L <0.056 (<LOQ, controls), 0.28, 0.53, 1.0, 2.2 and 4.7 ng ai/L	One composite sample (from both replicates) was analyzed for bifenthrin concentration at 0 and 96 hours. Nominal concentrations were 50% of the next higher concentration Concentrations were stable during the 96-hour study. 100% mortality was observed in the highest two concentrations after 96 hours
Solvent (type, percentage, if used): *Solvents should not exceed 0.5 ml L for static tests or 0.1 ml L for flow-though tests. OECD recommends that the solvent not exceed 100 mg L.*	Acetone, 0.050 mL/L	
Lighting: *EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD: optional light-dark cycle or complete darkness.*	16 hrs light/8 hrs dark, with 15- to 30-minute transition periods. Lighting intensity was 260 to 350 lux.	
Recovery of the chemical: Frequency of determination LOD: LOQ:	99.2 to 118% of nominal 0 and 96 hours Not reported 0.050 to 0.056 ng ai/L	Recoveries of QC samples fortified at 0.133, 1.00 or 4.00 ng/L and analyzed concurrently with the test samples.
Positive control {if used, indicate the chemical and concentrations}:	N/A	
Other parameters, if any:	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects/toxicity symptoms	Mortality Sublethal effects	
Observation intervals:	0, 24, 48, 72, and 96 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any:	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

In the negative control, solvent control, and mean-measured 0.28, 0.53, 1.0, 2.2 and 4.7 ng ai/L treatment levels, mortality averaged 0, 0, 0, 15, 25, 85 and 80%, respectively, after 24 hours; 0, 0, 0, 30, 70, 95 and 100%, respectively, after 48 hours; and 0, 0, 5, 55, 85, 100 and 100% respectively, after 72 hours. The 24- through 72-hour LC_{50} values and associated 95% C.I. were not reported.

After 96 hours of exposure, mortality averaged 0, 0, 5, 55, 100, 100 and 100% in the negative control, solvent control, and mean-measured 0.28, 0.53, 1.0, 2.2 and 4.7 ng ai/L treatment levels, respectively. The reported 96-hr LC_{50} (with 95% C.I.) was 0.50 (0.43 to 0.59) ng ai/L based on mean-measured concentrations.

Table 3: Effect of Bifenthrin on Mortality in *Hyalella azteca*.[a]

Mean-measured (and Nominal) Concentrations (ng ai/L)	# of Amphipods	Observation Period							
		24 hours		48 hours		72 hours		96 hours	
		No. Dead	% Mortality	No. Dead	% Mortality	No. Dead	% Mortality	No. Dead	% Mortality
Neg. control	20	0	0	0	0	0	0	0	0
Solv. control	20	0	0	0	0	0	0	0	0
0.28 (0.25)	20	0	5	0	0	1	5	1	5
0.53 (0.50)	20	3	15	6	30	11	55	11	55
1.0 (1.0)	20	5	25	14	70	17	85	20	100
2.2 (2.0)	20	17	85	19	95	20	100	20	100
4.7 (4.0)	20	16	80	20	100	20	100	20	100
LC_{50} (95% C.I.), ng ai/L		Not reported						0.50 (0.43 to 0.59)	

a Data were obtained from Table 4 on page 27 of the study report.

B. SUB-LETHAL TOXICITY ENDPOINTS:

No adverse effects were observed among *Hyalella* exposed at the control, solvent control, or mean-measured 0.28 ng ai/L levels. All amphipods were noted as swimming erratically at the (mean-measured) 4.7 ng ai/L treatment level at test initiation. Lethargy and/or immobility were observed at the ≥1.0 ng ai/L treatment levels by 24 hours and at the 0.53 ng ai/L level at 72 and 96 hours.

C. REPORTED STATISTICS:

The 96-hour LC_{50} and 95% confidence intervals were calculated using the Trimmed Spearman-Kärber Method via CETIS™ (2011) statistical software.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed the 96-hour mortality data using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/2014. Analyses were conducted using the negative control only and the α level was 0.05. All toxicity values were based on the mean-measured concentrations.

96-hour
LC_{50}: 0.493 ng ai/L 95% C.I.: 0.419-0.580 ng ai/L
Probit Slope: 7.32 95% C.I.: 4.18-10.5

NOAEC: 0.28 ng ai/L Based on lethargy and erratic swimming at 0.53 ng ai/L

Endpoint(s) affected: mortality, sub-lethal effects
Most sensitive endpoint(s): mortality

E. STUDY DEFICIENCIES:

Although data were available, the 24- through 72-hour LC_{50} values and associated 95% C.I. were not reported. In addition, the amphipods were fed throughout the study. There were no other significant deficiencies noted from U.S. EPA OCSPP *draft* Guideline No. 850.1020 (1996).

F. REVIEWER'S COMMENTS:

The reviewer's results were based on the probit analysis, whereas the study author used the Trimmed Spearman-Kärber method. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Exposure solutions and QC samples were analyzed for bifenthrin using gas chromatography in conjunction with mass selective detection (GC/MSD) based on methodology validated as Smithers Viscient. The method validation was conducted prior to the initiation of the definitive study and established an average recovery of 107 ± 9.44% from freshwater.

The experimental phase of the definitive test was conducted March 21 to 26, 2013.

G. CONCLUSIONS:

This study **scientifically sound** and is classified as **acceptable** form use in Agency risk assessments. After 96 hours of exposure, mortality averaged 0, 0, 5, 55, 100, 100 and 100% in the negative control, solvent control, and mean-measured 0.28, 0.53, 1.0, 2.2 and 4.7 og ai/L treatment levels, respectively. The 96-hr LC_{50} (with 95% C.I.) was 0.493 (0.419 to 0.580) ng ai/L. Erratic swimming followed by lethargy and/or immobility were observed in (several to all) amphipods from the ≥0.53 ng ai/L levels during the study.

96-hour

LC_{50}: 0.493 ng ai/L	95% C.I.: 0.419-0.580 ng ai/L
Probit Slope: 7.32	95% C.I.: 4.18-10.5

III. REFERENCES:

Dix, M.E. 2013. Method Validation for Eight Pyrethroids in Freshwater by Gas Chromatography using Mass Selective Detection with Negative Ionization and Liquid Chromatography with Mass Spectrometry. Smithers Viscient, Wareham, MA. Study No. 13656.6174.

Ives, M. 2011. CETIS™, Comprehensive Environmental Toxicity Information System™, User's Guide. Tidepool Scientific Software, McKinleyville, CA.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. *Water Research* 1:20-29.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Research* 3:793-821.

CETIS Summary Report

Report Date: 09

Jul-15 05:13 (p 1 of 1)

Batch Note: Sample Note: PC Code 128825 MRID 49552201

Point Estimate Summary

Analysis ID	Endpoint	Level	ng ai/L	95% LCL	95% UCL	TU	Method
12-8479-	96h Mortality Rate	LC5	0.294	0.19	0.359		Linear Regression (MLE)
		LC10	0.33	0.23	0.393		
		LC15	0.356	0.261	0.419		
		LC20	0.379	0.287	0.442		
		LC25	0.399	0.312	0.463		
		LC40	0.456	0.378	0.529		
		LC50	0.493	0.419	0.58		
00-3417-	96h Mortality Rate	LC50	0.503	0.429	0.591		Trimmed Spearman-Karber

96h Mortality Rate Summary

C-ng ai/L	Control Type		Mean	95%	95%	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	2	0	0	0	0	0	0	0		
0	Negative Control	2	0	0	0	0	0	0	0		
0.28		2	0.05	0	0.685	0	0.1	0.05	0.0707	141.0%	
0.53		2	0.55	0	1	0.5	0.6	0.05	0.0707	12.9%	
1		2	1	1	1	1	1	0	0	0.0%	
2.2		2	1	1	1	1	1	0	0	0.0%	
4.7		2	1	1	1	1	1	0	0	0.0%	

96h Mortality Rate Detail

C-ng ai/L	Control Type	Rep 1	Rep 2

96h Mortality Rate Detail

C-ng ai/L	Control Type	Rep 1	Rep 2
0	Solvent Blank	0	0
0	Negative Control	0	0
0.28		0	0.1
0.53		0.6	0.5
1		1	1
2.2		1	1
4.7		1	1